Vagal Afferents, Sympathetic Efferents and the Role of the PVN in Heart Failure.

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ABSTRACT

Sympatho-excitation is a characteristic of cardiovascular disease including heart failure (HF). The paraventricular nucleus of the hypothalamus (PVN) is an important site for central integration of sympathetic outflow. Atrial volume receptors (AVRs) in the wall of the right atrium transduce cardiovascular variables (pressure/volume) into an input that is integrated centrally, in for example, the PVN. Descriptions of the location and structure of the AVRs as well as the molecular mechanism initiating transduction remain scarce, nevertheless preautonomic neurons of the PVN have been consistently identified as making a significant contribution to the sympatho-excitation evident in HF. Furthermore, excitatory and inhibitory interactions within the PVN determine sympathetic tone. A nitric oxide dependent GABAergic inhibition sets the prevailing sympathetic output from the PVN, which in HF becomes dysregulated. Inflammation and oxidative stress have been recognised as possible triggers to the disinhibition. The actions of proinflammatory cytokines and reactive oxygen species in relation to the signalling pathways, which are important in generating sympathetic tone are discussed, as well as the contribution these might make to abnormal control of the sympathetic nervous system in cardiovascular disease.

Key words: atrial volume reflex arc, atrial volume receptors, sympathetic efferent output, paraventricular nucleus of the hypothalamus, heart failure.

1. INTRODUCTION

Cardiovascular regulation is a key component in mammalian survival. The process of maintaining adequate organ perfusion in the face of a continually varying metabolic demand requires integration of a multitude of "signals" to produce a co-ordinated cardiovascular output. This is achieved in part by defined reflex responses that react to disturbances not only in cardiovascular variables, (pressure, volume) but also to neuroendocrine stimuli. Specific neuronal networks within the autonomic control centres in the hypothalamus and medulla produce an adaptive neurohumoral response to match the organ perfusion demand.

A key reflex regulating cardiovascular function, in particular blood volume homeostasis, involves the atrial volume receptors (AVR's), the paraventricular nucleus of the hypothalamus (PVN) and the autonomic nervous system. This reflex underpins normal water and electrolyte regulation, but in cardiovascular disease especially heart failure (HF) following a myocardial infarction (MI), this reflex becomes dysfunctional. In this review we intend to highlight the recent advances in our understanding of this reflex and its possible role in the pathophysiology of cardiovascular disease.

2. Central Control of Sympathetic Efferents

Control of the autonomic nervous system, in particular the sympathetic nervous system, originates in several areas of the brain including the nucleus tractus solitarii (NTS), rostral ventrolateral medulla (RVLM) and the PVN (Guyenet, 2006). The role of the PVN in cardiovascular homeostasis and involvement in the generation of abnormal sympathetic outflow in HF has been extensively reviewed (Pyner, 2009,

2014). There is general agreement that the PVN receives visceral cardiovascular signals from the NTS and then using its direct and indirect projections influences sympathetic outflow through the sympathetic preganglionc neurons in the spinal cord (Figure 1). A balance between the excitatory actions of glutamate and angiotensin II (ANGII) and the inhibitory actions of nitric oxide (NO) and gamma-aminobutyric acid (GABA) determine output from the preautonomic neurons of the PVN. At rest the prevailing state of these neurons is tonic inactivity determined by an NO mediated GABAergic inhibition. Nitric oxide in the brain is generated by the enzymes of the nitric oxide synthase (NOS) family. In HF the preautonomic neurons become disinhibited leading to increased sympathetic activity. The fundamental mechanism underpinning sympatho-excitation in HF involves loss of NOS signalling (Biancardi et al., 2010; Wang et al., 2014). Inflammation and oxidative stress are being touted as novel mechanisms by which the loss of NOS signalling may occur leading to sympatho-excitation.

Again, the role neurotransmitters play in abnormal sympatho-excitaion in HF has been extensively reviewed (Pyner, 2009, 2014). To focus on NO, within the brain, all three isoforms of NOS, inducible (iNOS), endothelial (eNOS) and neuronal (nNOS) contribute to the production of NO (Stern, 2004). In healthy animals, PVN NO acts to suppress sympathetic activity by inhibiting the excitatory neurotransmitter glutamate receptor (NMDA) and by facilitating tonic GABAergic inhibition. Glutamatergic activity also leads to NO production by nNOS creating a negative feedback loop. Nitric oxide release is tightly controlled to ensure specificity and avoid toxicity (Alderton et al., 2001) and this mechanism appears to be dysfunctional in HF. The signalling proteins CAPON (carboxy-terminal PDZ ligand-PSD95/Discs

large/zona occludens-1 of nNOS) and PIN (protein inhibitor of nNOS) regulate NO generation. Activation of the NMDA receptor leads to Ca^{2+} entry into the cytoplasm of the preautonomic neuron. The enzyme nNOS forms a complex with the polysynaptic density protein PSD95 domain of the NMDA receptor that places the nNOS enzyme in close proximity to the entering Ca^{2+} promoting Ca^{2+}-calmodulin-induced activation of nNOS and thus production of NO. Conversely, ANGII activation of the ANGII type 1 receptor (AT1R) results in the expression of CAPON and PIN, whereby the CAPON competes with PSD95 for the binding of nNOS while PIN destabilises nNOS homodimers (Sharma et al., 2011; Sharma et al., 2013). Maintaining the balancing of excitatory and inhibitory influences on preautonomic neurons for normal control shows that ANGII binding to AT1R potentiates neuronal excitability but this effect is then modulated by the NO-GABAergic feedback system (Li et al., 2003). However, in HF, the actions of ANGII are upregulated thereby allowing PIN to interfere with the production of NO and remove the tonic inhibition of the preautonomic neuron (Figure 2).

The normal catalytic activity of nNOS requires homodimerisation with the cofactor endothelial tetrahydrobiopterin (BH_4) binding to stabilise the dimer. This configuration allows electrons to transfer from the oxygenase domain of one monomer to the reductase domain of another monomer. Neuronal NOS activation without proper BH_4 binding uncouples normal electron transfer to produce superoxide (Alkaitis & Crabtree, 2012; Figure 3). The availability of BH_4 in HF is known to be impaired in the endothelium of these animals, however it remains to be seen if a similar reduction in BH_4 availability is a characteristic of the central nuclei involved in cardiovascular regulation (Schmidt & Alp, 2007).

2.1 Inflammation and reactive oxygen species

While transcriptional, translational and posttranslational mechanisms in the signalling pathway for nNOS control are evident in cardiovascular pathologies, the question remains what is/are the trigger(s) for the decoupled control? These effects are probably related to increased "oxidant stress" linked to increased reactive oxygen species (ROS) and pro-inflammatory molecule production. The generation of ROS is a normal by-product of cellular metabolism and is tightly regulated by antioxidant enzymes (Zimmerman & Davisson, 2004). Pro-inflammatory cytokines (PICs) increase in the brain, heart and plasma within minutes of a myocardial infarction (MI). Some are transported into the hypothalamus and brainstem via the circumventricular organs, however the appearance of PICs in the brain after MI is independent of blood-borne cytokines and it has been shown that cardiac sympathetic afferents activated by myocardial ischaemia signal the brain to increase cytokine production (Francis et al., 2004). In the latter study increases in tumor necrosis factor (TNF)-α and interleukin (IL)-1β were confined to the hypothalamus suggesting it was not a generalized central response to myocardial injury. Given the importance of the hypothalamus in volume regulation, stress responses and sympathetic drive it is likely that this signal to the brain has a specific function. Initially it may be protective, but damaging in the longer term. In the PVN activation of the membrane-bound enzyme complex NADPH oxidase (Nox) is a major source of ROS. In HF, Nox appears to be of some importance as Nox4 (the major isoform expressed in the PVN) is associated with sympatho-excitation and impaired cardiac function (Guggilam et al., 2011; Infanger et al., 2010).

Elevated TNF in the PVN and ventrolateral medulla alters the production of superoxide and NO leading to sympatho-excitation and fluid imbalance in HF mice (Guggilam et al., 2011). In addition, blockade/deletion of TNF in the PVN and ventrolateral medulla attenuated neurohormonal excitation elicited by HF. In TNF-α - knockout mice, or wild type mice where TNF was pharmacologically blocked, there was a reduction in the production of PICs and ROS. The HF-induced reduction of nNOS in these key regulatory sites was decreased, thereby preserving NO levels and curtailing neurohormonal excitation. Maintaining NO levels also reduced the formation of potentially damaging peroxynitrite. Therefore, TNF and nNOS could well trigger decoupled control.

Angiotensin II could also be involved as ANGII infusion induces imbalances between excitatory and inhibitory neurotransmitters and pro- and anti-inflammatory cytokines in the PVN (Kang et al.,2014). Paraventriuclar hypothalamic inhibition of ANGII with the ANGII converting enzyme inhibitor enalaprilat restores the neurotransmitters and cytokines in the PVN and reduces ANG II- induced hypertension and cardiac hypertrophy. In addition, blockade of NF-κB by a number of different strategies can all diminish the production of superoxide and peroxynitrite within the PVN in response to systemic ANGII infusion (Kang et al., 2009; Cardinale et al., 2012). The NF-κB activation sites may be localised to the proopiomelanocortin or POMC neurons which project to the PVN (Purkayastha et al., 2011).

165 **2.2 Other mediators**

166 A recent study explored factors regulating the expression of the chemokine SDF-1
167 (stromal cell-derived factor-1) in the PVN and the mechanisms leading to its
168 sympatho-excitatory effects (Wei et al., 2014). This novel cytokine and its receptors
169 have been found expressed by neurons and glial cells in cardiovascular autonomic
170 regions of the brain, including the PVN. Both TNF-α and ANGII were identified as
171 drivers of SDF-1 expression in PVN and their cardiovascular and sympathetic effects
172 depended upon SDF-1-mediated activation of the p44/42 MAPK signalling pathway.
173 Previous work indicates a role for SDF-1 as a mediator of neurohumoral excitation in
174 HF rats (Wei et al., 2012). How MAPK signalling leads to sympatho-excitation is not
175 known. Phosphorylated MAPKs have nuclear and cytoplasmic effects (Turjanski et
176 al., 2007) and may have both long- and short-term effects on the excitability of PVN
177 neurons. They act on nuclear transcription factors including NF-κB (known to drive
178 the transcription of a range of inflammatory mediators) and they may augment the
179 production of pro-hypertensive renin angiotensin system components.
180 Interestingly, MAPK signalling may also modulate the transient outward potassium
181 current that normally restrains neuronal excitability in cardiovascular related central
182 nuclei (Gao et al., 2010). The delay in neuronal excitation following
183 hyperpolarization induced by GABA is largely determined by the expression of the
184 potassium channel subunits Kv4.2 and Kv4.3, however whether this is the case in the
185 PVN remains to be tested.
186 Another interesting component to the inflammation-oxidative stress mechanism is
187 the role of Toll- like receptors (TLRs) in particular TLR4. Toll-like receptor 4 is a
188 signalling receptor involved in the innate immune response (Takeda & Akira, 2001).

189 Of the 13 TLRs identified in mammals, TLR4 has been implicated in cardiovascular
190 disease (Baumgarten et al., 2001). The TLR recognises specific damage associated
191 molecular patterns (DAMP) with high mobility group box -1 (HMGB1) being the most
192 important DAMP implicated in various inflammatory conditions. The TLR4 receptor
193 is expressed in microglial cells, the immune cell of the brain. Microglial activation is
194 related to injury and infection, which results in the release of PICs and ROS and
195 importantly ANGII stimulation appears to facilitate the inflammatory response in the
196 PVN (Shi et al., 2010). Biancardi and colleagues using mice (Biancardi et al., 2016)
197 have demonstrated a functional interaction between AT1R and TLR4 in mediating
198 ANGII-dependent microglial activation and oxidative stress within the PVN. Similarly,
199 Dange et al., (2014) recently demonstrated that ANGII-infused hypertensive rats had
200 increased TLR4 expression in the PVN and central blockade of these receptors
201 delayed the progression of hypertension. These authors provided evidence that
202 TLR4 inhibition attenuated ANGII-induced hypertension by down-regulation of
203 myocardial PICs and reducing circulating levels of plasma noradrenaline indicative of
204 a reduction in sympathetic activation. They also showed that activation of TLR4
205 could induce the sympatho-excitation observed in hypertension, possibly due to
206 increased PICs. The same group has recently reported that TLR4 blockade is similarly
207 protective in the spontaneously hypertensive rat (SHR), a model of human essential
208 hypertension (Dange et al., 2015). The SHR animals had increased levels of TLR4 in
209 the PVN localised to neurons and microglia. Blockade of TLR4 within the PVN
210 attenuated both the increase in blood pressure and cardiac hypertrophy in the SHR.
211 In addition, TLR4 inhibition in the PVN reduced pro-inflammatory cytokines, iNOS,
212 and transcription factor NF-κB activity within the PVN itself, whereas levels of the

anti-inflammatory cytokine IL-10 in the PVN were increased. The damage associated molecular pattern, HMGB1 may be a mediator of the changes seen in hypertensive animals, since there was an increase in HMGB1 levels both in the PVN and in the plasma. Thus to understand pathological sympatho-excitation, inflammation and oxidative stress effects need to be investigated further.

3. Cardiac vagal afferents

The previous section has described knowledge of triggering factors that impact on the excitability of PVN-presympathetic neurons and how these might be involved in cardiac pathologies. However, where we lack detail is the afferent input to the brain from sensory mechanisms in the periphery. Relatively little progress regarding structure, function and location of these inputs has occurred since cardiac reflexes were extensively reviewed by Hainsworth 25 years ago (Hainsworth, 1991). It is important to again focus attention on cardiac afferents because we have since learned that resetting of the gain of atrial volume receptors, making them less sensitive to local signals, occurs in various strains of hypertensive rats as well as in a sheep model of heart failure. Also in pregnancy there is a reduction in the sensitivity of these receptors (Hines et al., 2005; Ricksten et al., 1979; May et al., 2013).

While cardiovascular pathologies might indicate an involvement of the afferent arm, to date, much is still unknown regarding its normal mode of functioning let alone its possible contribution to heart disease. The volume receptors are particularly challenging, their location making them less accessible than other receptors. Studies from as far back as the 1950's described the electrophysiological properties of receptors at the veno-atrial junction that were sensitive to volume changes (Paintal,

1953). Their morphology was examined initially using classic neuronal stains (Woollard, 1926; Coleridge et al., 1957; Holmes, 1957) and later electron microscopy (Tranumjensen, 1975). However until recently, information on their exact location and distribution has been lacking; as has any understanding of the molecular machinery underpinning their function. Neuroanatomical studies combined with powerful new imaging techniques are now providing a means to visualize these receptors. Furthermore, understanding of the proteins and processes involved in detecting mechanical stimuli in mammalian systems is now making progress.

There is still greater uncertainty about vagal afferents arising from the ventricles, despite numerous electrophysiological studies (Hainsworth 1991). In particular, the physiological stimuli to which these receptors respond remains to be determined as it is technically very difficult to isolate responses that arise exclusively from activation of ventricular vagal afferents. It may be that the atrial and ventricle afferents are both chemo- and mechanosensitive. For these reasons and because the research of our own group focuses on volume sensing in the veno-atrial junction, ventricular vagal afferents are not included here. Nevertheless current thinking on the molecules and proteins involved in mechanotransduction is likely to apply to afferents arising from both.

3.1 Distribution and structure

The sensory atrial volume receptors are said to be in the subendocardial tissue mainly at the junction of the veins with the atria and in the appendages (Nonidez, 1937; Coleridge et al., 1957; Coleridge et al., 1964; Floyd et al., 1972). Despite a number of detailed histological studies of nerves in the endocardium of several

mammalian species the structure of the AVRs has not been unequivocally determined (Hainsworth, 1991). Nonetheless, two structures have been described, complex unencapsulated endings and end nets. Myelinated nerves supply complex unencapsulated endings and there is evidence that these are mechanoreceptors (Holmes, 1957; Coleridge et al., 1973; Tranumjensen, 1975). End nets consist of a fine network of fibres that cover the entire endocardial surface of the heart, including the ventricular endocardium (Woollard, 1926).

There are few publications where more recent imaging techniques have been employed. A confocal and fluorescence microscopy study of the human heart found no evidence for an end net but did describe myelinated fibres of two types in the atrial endocardium (Marron et al., 1995). The fibres were distinguished by the size of the area covered by their terminals, one type giving rise to terminals over an area roughly three times that of the other type. The fibres were mostly tyrosine hydroxylase or neuropeptide Y positive, traditionally considered as efferents; however there is evidence that primary sensory neurons may express these markers (Katz, 1987; Czyzyk-Krzeska, 1991; Finley, 1992).

Another study in the rat using anterograde labelling of cell bodies within the nodose ganglion and confocal microscopy distinguished “flower-spray” and “end-net” terminals (Cheng et al., 1997). These authors proposed that the flower-sprays resemble early descriptions of complex unencapsulated endings. However, they did not differentiate between myelinated and non-myelinated fibres and the lack of clarity concerning the morphology of these receptors remains. Indeed there may be important differences between species since unencapsulated endings have not been described in rats (Kaufman et al., 1981), apart from the “flower-sprays” alluded to

285 above (Cheng et al., 1997). It has also been suggested that the morphological
286 differences, which appear to exist between unencapsulated endings and end-nets
287 are quantitative rather than qualitative and that end nets should in fact be
288 considered as a variation within the group of unencapsulated endings (Hainsworth et
289 al., 1991). Comprehensive studies of this sort describing in detail the morphology
290 and location of these endings is essential to extending our understanding of how
291 they operate.
292 Cheng and colleagues (Cheng et al., 1997) showed for the first time vagal afferent
293 nerve endings with dense pericellular varicose terminals around small intensely
294 fluorescent (SIF) cells in each ganglion of the cardiac plexuses as well as retrogradely
295 labelled neurons in the ganglia. These observations lend support for the presence of
296 SIF cells within the intrinsic plexuses of the heart together contributing via a
297 selection of neurochemical modulators to both local regulation and more
298 widespread effects (Eranko & Eranko, 1977). Polymorphic endings contacting both
299 cardiomyocytes and connective tissue in the endocardium, which could account for
300 some of the intermediate AB-type discharges (see section 3.2 Function) noted in
301 electrophysiological studies have also been reported (Cheng et al., 1997). More
302 recently vagal intramuscular array afferents in gastrointestinal smooth muscle have
303 been shown to contact interstitial cells of Cajal (Powley and Phillips, 2011).
304 Moreover, axons positive for various efferent and afferent markers (tyrosine
305 hydroxylase, vesicular acetylcholine transporter, nitric oxide synthase and calcitonin
306 gene-related peptide) meet in the intramuscular array- interstitial cells of Cajal
307 complexes in the gastrointestinal wall. This architecture is likely to be integral to the
308 way these mechanoreceptors work and similar arrangements can be expected for

309 cardiac vagal afferents. With this in mind it will be important to precisely locate
310 vagal afferents in situ and determine their relationship with neighbouring cells of all
311 types. Interestingly a new type of interstitial cell has been described in the heart
312 (Popescu and Faussone-Pellegrini, 2010). Initially these cells were called interstitial
313 cells of Cajal -like cells, but more recently they have been given the name telocytes.
314 However, though they may be a type of fibrocyte/fibroblast rather than a completely
315 novel cell type, there is evidence that they are indeed distinct (Bei et al., 2015). They
316 are distinguished by the presence of caveolae and extremely long, thin cell body
317 extensions termed telopods, which can only be visualised using electron microscopy
318 or specialised light microscopy on ultra-thin tissue sections. They are present in
319 myocardial sleeves of human pulmonary veins and all three layers of the cardiac wall,
320 often in close association with capillaries and nerves (Gherghiceanu et al., 2008).
321 They may play a role in chemo-mechanical transduction, though this remains to be
322 determined. Interestingly they have also been shown in the capsule surrounding
323 muscle spindles where they could have both a passive mechanical involvement as
324 well as a neurosecretory role (Diaz-Flores et al., 2013).
325
326 **3.2 Function**
327 Atrial receptors have been typically classified as either A-type myelinated or B-type
328 according to their pattern of discharge in relation to the atrial pressure wave (Paintal,
329 1953). Broadly, A-type receptors respond to atrial contraction while B-type are
330 stimulated by atrial filling and are therefore considered to be the volume receptors.
331 However, intermediate AB-type discharges have also been described, suggesting that
332 there may be only one type of receptor with the different discharge patterns

333 determined by the location of the receptor rather than any real dissimilarity in
334 structure (Kappagoda et al., 1976). These early experiments were carried out in cats
335 and dogs and recordings were from myelinated afferents postulated to arise from
336 unencapsulated endings. Two subtypes of unmyelinated atrial C-fibres, high
337 frequency and low frequency receptors have been described in cats (Coleridge et al.,
338 1973; Thorén, 1977) and rats (Thorén et al., 1979). Slowly adapting and rapidly
339 adapting have also been described in the rat and these fibres might correspond to
340 the end net (Mifflen & Kunze, 1982, 1984). With these varying discharge
341 descriptions there is therefore a clear need to be able to reliably identify AVRs so
342 that the electrophysiological and morphological/molecular characteristics can be
343 correlated.
344
345 **3.3 Molecular Characterisation**
346 The identification of mechanosensitive channels in mammalian systems remains
347 elusive. However, two channel protein families in particular are candidates: the
348 Epithelial Na Channel/Degenerin/Acid Sensing Ion Channel (ENaC/Degenerin /ASIC)
349 and Transient Receptor Potential (TRP) families (Delmas et al., 2011). Recent studies
350 have indicated a role for amiloride-sensitive channels (i.e. likely to be related to the
351 ENaC/Degenerin/ASIC family) in mechanotransduction in rat muscle spindles (Simon
352 et al., 2010). Transient Receptor Potential proteins have been implicated in
353 mechanosensation in heart as well as other tissue (Inoue et al., 2009).
354 The γ subunit of ENaC is expressed in baroreceptor nerve terminals innervating the
355 aortic arch and carotid sinus in mice (Drummond et al., 1998). The ASIC1, 2 and 3
356 ion channels were found in aortic baroreceptor neurons in the nodose ganglia and

their terminals in the aortic arch (Lu et al., 2009). This same study showed that ASIC2 null mice had an impaired baroreceptor reflex and developed hypertension, lending support to the idea that compromised mechanosensing of blood pressure could underlie the disturbed autonomic drive seen in heart failure and hypertension. Lee and colleagues provide evidence for a role of ASIC3 in blood volume control in mice, such that blood volume expansion-induced urine flow, neural activation, and atrial natriuretic peptide (ANP) release were reduced in ASIC3 -/- knockout mice compared with controls (Lee et al., 2011). They showed ASIC3-IR co-localising with Calcitonin Gene Related Protein immunoreactivity (CGRP-IR) on nerve terminals in the veno-atrial junction area. However, gadolinium (a non-selective blocker of stretch- activated ion channels) reduced these blood volume expansion effects both in ASIC3 -/- and ASIC3 +/+ control mice. Therefore, the gadolinium sensitivity cannot be exclusively due to blockade of ASIC3.

Broad ranges of stimuli have been found to activate the TRP family of ion channels, including direct activation by heat, cell swelling or mechanical perturbations (Ramsey et al., 2006). The TRP channels are widely expressed in the cardiovascular system and there is increasing evidence for their importance in heart disease (Inoue et al., 2006; Watanabe et al,. 2008; Inoue et al., 2009, Feetham et al., 2015). So far focus has mostly been on their role in the maintenance of myogenic tone, and vascular injury and remodelling following insult (Stiber et al., 2012). Few studies have been undertaken to look for TRP expression in mechanosensory organs and endings despite the fact that they are considered to be strong candidates as the elusive mechanosensitive channels in mammals (Delmas et al., 2011). Nevertheless there are some pointers: a pressure-induced calcium influx with characteristics compatible

381 with TRP sensitive channels has been described in baroreceptor neurons from
382 nodose ganglia of rats (Sullivan et al., 1997). The TRPC1 and TRPC3-5 channels are
383 present not only in the somata of nodose ganglion sensory neurons but also in the
384 peripheral axons and mechanosensory endings that terminate as mechanosensitive
385 receptors in the aortic arch of the rat (Glazebrook et al., 2005). The TRPC1 channel
386 has been shown to contribute to light-touch sensation and mechanical responses in
387 low-threshold cutaneous sensory neurons innervating Merkel cells in mice (Garrison
388 et al., 2012). The TRPV4 channel is also present in rat Merkel cells where it may play
389 a dual role both as a mechanotransducer and in neurosecretory granule exocytosis
390 (Boulais et al., 2009). Furthermore, TRPV4 has been implicated in mechanosensation
391 in inner ear hair cells, but this remains to be proven (Mutai and Heller, 2003). The
392 TRPV4 selective activator 4α-phorbol 12,13-didecanoate results in dose-dependent
393 decreases in blood pressure (Gao et al., 2009). We have recently provided the first
394 evidence that in rat heart the TRP channels, TRPC1 and TRPV4 are expressed in
395 sensory endings found in regions of veno-atrial endocardium where AVRs are located
396 (Fig 4, 5). The TRPC1 and TRPV4-IR co-localises with synaptophysin, a marker of
397 neuronal synaptic-like vesicles and CGRP a marker for sensory neurons (Shenton and
398 Pyner, 2014). Synaptic-like vesicles have commonly been described in
399 mechanosensory endings of vertebrate and invertebrate animals (Katz, 1966) and
400 there is evidence that they play a role in regulating their excitability (Bewick et al.,
401 2005).
402 Interestingly, one area where the role of TRPV4 as a mechanosensitive channel has
403 been investigated involves osmotic stimuli and autonomic regulation (Benfenati et
404 al., 2011). Changes in osmolality have been shown to elicit cellular responses that

involve TRPV4-mediated elevations of intracellular calcium (Liedtke et al., 2003) with activation of intermediate (IK)- and small (SK)- conductance calcium-activated potassium channels (Sonkusare et al., 2012). The hypothalamus expresses both TRPV4 (Guler et al., 2012) and SK channels (Gui et al., 2012) and genetic deletion of TRPV4 channels results in blunted autonomic response to osmotic disturbances (Liedtke and Friedman, 2003). With this in mind a recent study has shown that a hypo-osmotic stimulus hyperpolarises parvocellular neurons of the PVN through a TRPV4–SK ion channel mechanism (Feetham et al., 2015).

3.4 Integration with other systems

At its simplest mechanotransduction is the conversion of a physical deflection (the stimulus) into a neural signal. Understanding how this is achieved is essential, however in a whole behaving organism this is only one small component contributing to cardiovascular control. The local cellular environment will influence the transduction process. Output from AVRs may be subject to the influence of a range of neuromodulators and neuroendocrine factors (Antunes-Rodrigues et al., 2004). Atrial natriuretic peptide in particular is likely to play an important role, since systemic administration of ANP has been show to decrease renal sympathetic nerve activity (Lovick and Coote 1989; Yusof et al., 2009). This suggests that ANP may activate cardiac vagal afferents that inhibit the spinally projecting vasopressin neurons at their origin in the PVN (Yusof et al., 2009). There is evidence for sensory receptors in epicardium as well as endocardium. In human hearts, Marron et al., (1995) found terminals on both sides of the atrial appendages and some on the epicardial surface of the superior caval and pulmonary veins, where they could

429 respond to inflation of the lungs. Endings in the epicardium were often associated
430 with epicardial mesothelial cells suggesting the possibility that local
431 neuromodulators secreted by these cells might regulate afferent output. The SIF
432 cells (Eranko and Eranko 1977) reside within the cardiac ganglia alongside principal
433 neurons and the SIF cells seem to be innervated by vagal afferents rather than the
434 principal neurons (Cheng et al., 1997). The nature and role of SIF cells is not fully
435 understood, there is speculation that they may be chemosensory and/or
436 neurosecretory since they contain neurotransmitters and other neuroactive
437 substances. In addition they may be a type of interneuron in the intrinsic neural
438 network of the heart (Pauza et al., 2014). These observations provide the
439 anatomical evidence for "accessory cells" being able to contribute to neural
440 interactions and output, but further studies are needed to more precisely define
441 their role and mode of action.
442
443 **3.5 Role of atrial volume reflex in cardiovascular disease**
444 Improved understanding of how changes in returning blood volume are sensed and
445 how they influence cardiac output are timely and important. Resetting of atrial
446 volume receptors has been demonstrated in the SHR, the threshold pressure in the
447 left atrium at which renal nerve inhibition was elicited being higher in the
448 hypertensive animals compared with controls (Ricksten et al., 1979). Atrial volume
449 receptors appear to be less sensitive in both hypertension in rats (de Andrade et al.,
450 2008) and in a sheep model of heart failure (May et al., 2013). May and colleagues
451 focused on the effects of heart failure on cardiac sympathetic nerve activity. In
452 sheep and other species the reduction in renal sympathetic nerve activity in

response to activation of AVRs is severely impaired in heart failure. To date there is less information on the factors contributing to the increase in cardiac sympathetic nerve activity observed in heart disease. It has not so far been possible to carry out equivalent experiments in humans; nevertheless it has been shown that the sensitivity of the peripheral component of the volume-sensitive cardiopulmonary reflex is altered in elderly humans compared with younger controls (Salem 1969; Cleroux et al., 1989). Although results were contradictory with the first study showing an enhancement and the later investigation reporting impairment, important differences in the two studies may account for these apparent discrepancies (Crystal and Salem 2012). There is an ongoing debate over whether fluid re-distribution rather than accumulation is more important in heart failure (Dunlap and Sobotka 2013), an issue of clinical relevance when deciding whether heart failure patients are best treated using current decongestion strategies to reduce total body salt and water. One avenue to gain insights for this may come from pregnancy. Autonomic reflexes are attenuated during pregnancy and gestational alterations in central sites that regulate the efferent limb of the reflex have also been reported (Deng & Kaufman, 1995; Heesch & Rogers, 1995; Cork et al., 2016). Atrial volume receptor discharge is reduced during pregnancy and is accompanied with an increase in right atrial dimension to accommodate the increased blood volume without an increase in right atrial pressure (Hines and Hodgson et al., 2000; Hines et al., 2005). However, the reduced afferent discharge does not appear to be related to the atrial dimension-pressure change, which might argue for a mechanism within signal transduction being a candidate.

4. Conclusion

The control of the reflex circuit regulating cardiovascular homeostasis is complex. It is evident the maintenance of the centrally generated tonic sympatho-inhibition is dependent upon mechanoreceptors sensing cardiovascular status. However, our current understanding would indicate that signalling processes are major contributors to disturbed cardiovascular control in heart failure and hypertension. The triggers for these are beginning to be revealed and provide some insights.

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766

Figure 1: Schematic to show the components of the atrial volume reflex arc.

A. volume receptors in the right atria of the heart communicate via vagal afferents with the nucleus tractus solitarii (NTS). The NTS is reciprocally connected with the paraventricular nucleus of the hypothalamus (PVN). The PVN influences sympathetic outflow via connections with the RVLM and sympathetic preganglionic neurons in the spinal cord.

B. PVN directed NTS axons target at least four neuronal pools that are associated with cardiovascular control (1) preautonomic neurons (2) magnocellular neuronal nitric oxide (nNOS)-containing neurons (3) GABAergic interneurons connected to preautonomic neurons and (4) nNOS-interneurons bordering the PVN. (Pyner, 2014 with permission).

Abbreviations

PaDC -Parvocelular-dorsal cap

PaLM -Parvocellular-lateral magnocellular

PaVP -Parvocellular-medial parvicellular

PaV -Parvicellular-ventral part

3V -3rd ventricle

RVLM -rostral ventrolateral medulla

Figure 2

Proposed model for the down regulation of nNOS by posttranslational regulation in the PVN in HF. In HF, carboxy-terminal PDZ ligand of nNOS (CAPON) and PIN are overexpressed due to increased ANGII levels and AT1 receptors in the PVN. Increased CAPON competes with postsynaptic density (PSD)95 for binding to nNOS and sequesters nNOS therefore decreasing N-methyl-D-aspartic acid receptor (NMDAR)/PSD95/nNOS complexes. Binding of PIN to nNOS in HF destabilises nNOS dimers, which renders nNOS catalytically inactive by interfering with either the assembly or dimer stability. Inactive nNOS monomers are susceptible to ubiquitination and subsequent proteosomal degradation. This results in decreased levels of nNOS in the PVN of HF rats. A decreased level of nNOS reduces NO production in the PVN during HF causing an increase in sympathetic nerve activity (SNA). (Sharma et al., 2013 with permission).

Figure 3

Coupled nNOS (nNOS homodimer) produces NO, whereas uncoupled nNOS monomer produces superoxide.

A. nNOS uncoupling occurs during the conversion of nNOS homodimer to nNOS monomer. Two nNOS monomers are connected with the aid of Zn^{2+} connection (not shown), making the nNOS homodimer. BH4 strengthens the Zn^{2+} connection, maintaining the dimer form. In coupled NOS, an electron is transferred to L-arginine (L-Arg) producing NO and L-citrulline (L-Cit).

B. Electron from NADPH is transferred to O2 in the uncoupled nNOS in the absence of BH4, thereby producing superoxide.

Abbreviations

FMN/FAD	flavin mononucleotide/flavin adenine dinucleotide
NADPH/NADP	nicotinamide adenine dinucleotide phosphate (reduced)/ nicotinamide adenine dinucleotide phosphate
Fe	iron
CaM	calmodulin
BH4	tetrahydrobiopterin
nNOS	neuronal nitric oxide synthase

819 **Figure 4**

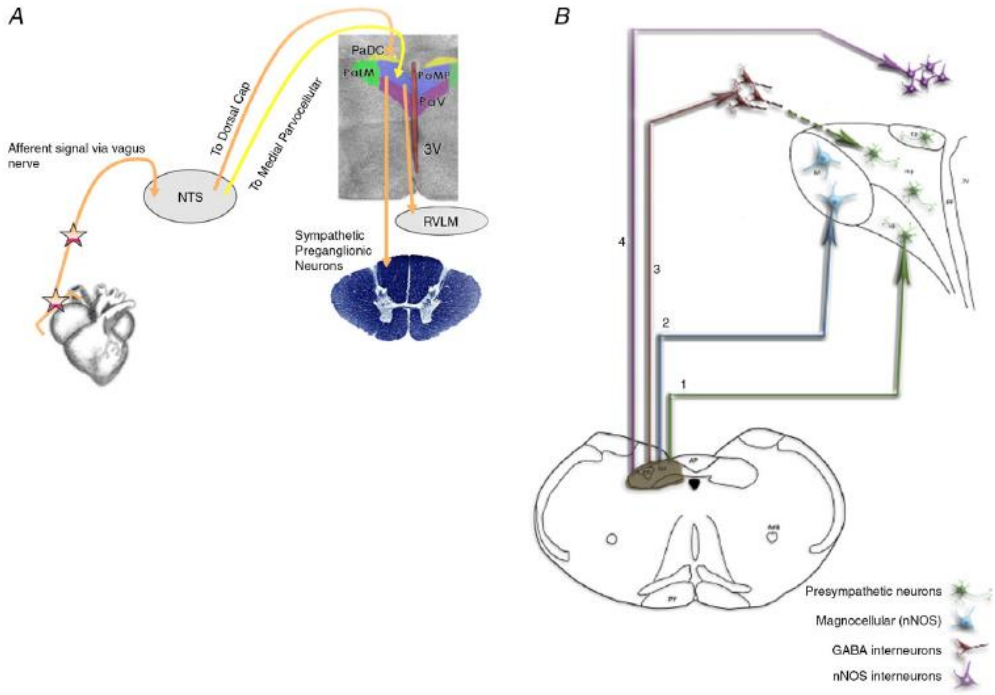
820 Mechanosensitive ion channels: Transient Receptor Potential Canonical 1 (TRPC1)
821 labelling in the endocardium. SYN immunoreactivity (SYN-IR) (short arrows, A) and
822 TRPC1-IR (long arrows, B) were both evident within the endocardium. Panel C is the
823 merge of A and B, TRPC1-IR coincided with SYN-IR labeling on nerve endings
824 (arrowheads, C). Panel C' is a 3-D Opacity image displayed as an isosurface to
825 demonstrate the concurrence and compartmentalisation of TRPC1 and SYN labeling
826 (black arrows, C'). (Shenton & Pyner, 2014 with permission).

827

828 **Figure 5**

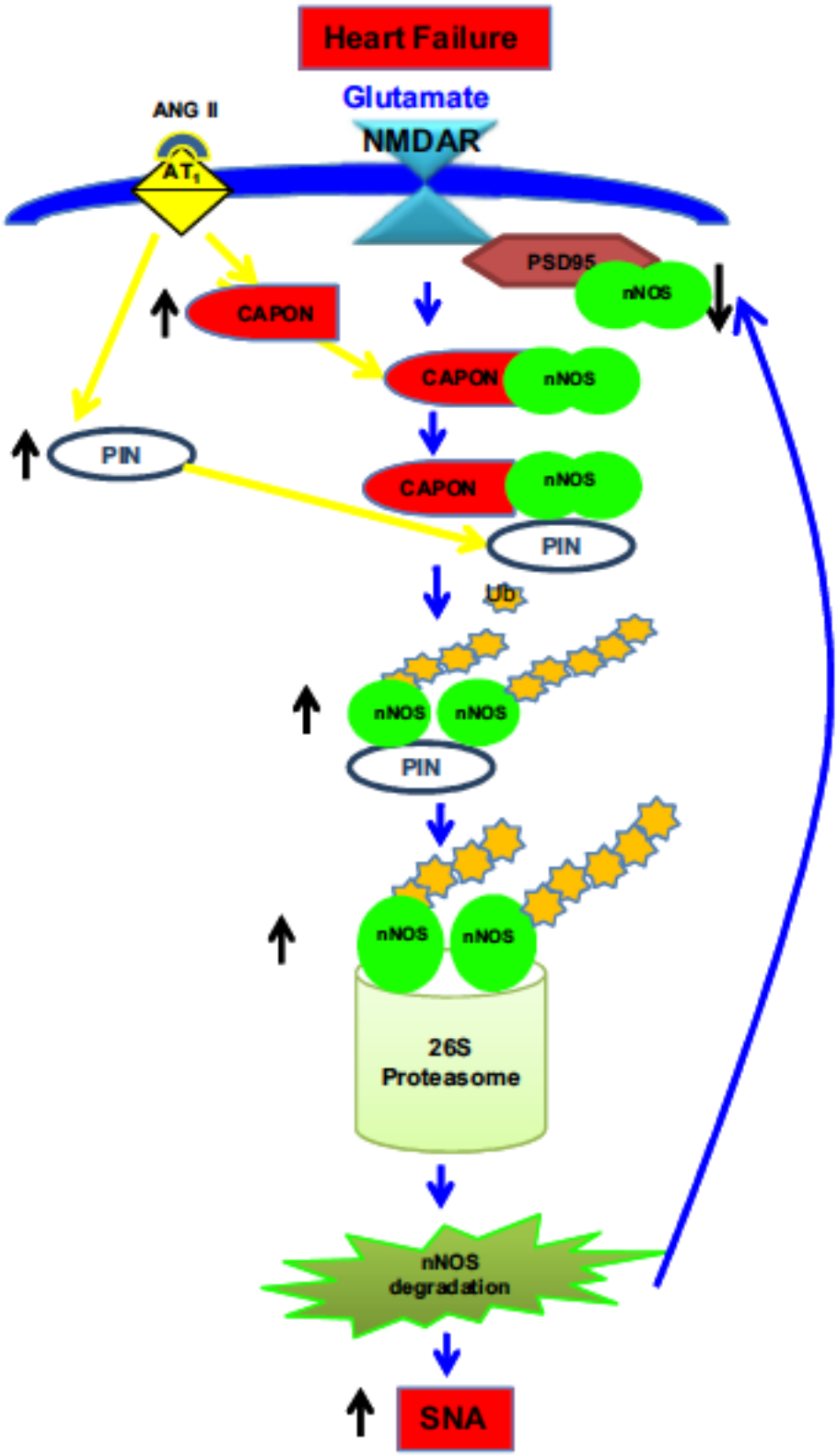
829 Mechanosensitive ion channels: Transient Receptor Potential Vanilloid4 (TRPV4)
830 labelling in endocardium and myocardium. TRPV4 immunoreactivity (TRPV4-IR)
831 (long arrows B, E) was widespread in the endocardium (ENDO) and also extended
832 into the myocardium (MYO). Nerve endings identified by SYN-IR (short arrows, A)
833 were co-labelled with anti-TRPV4 (arrowheads, C). Panel C' is the isosurface
834 presentation of a Volocity 3D slice to illustrate the close relationship between TRPV4
835 and SYN labeling (black arrows, C' indicate concurrent TRPV4-SYN labelling). CGRP-IR
836 was only rarely found in either endocardium or myocardium (short arrows, D).
837 However, on the occasions when it was present the endings were also TRPV4
838 positive (arrowhead, F). Panel F' is the isosurface presentation of a Volocity 3D slice
839 to illustrate the presence of CGRP labeling on TRPV4-positive endings (black arrows,
840 F' indicate dual labeling). The isosurface view F' is again indicative of anti-channel
841 and sensory nerve labelling occurring in distinct compartments within the same
842 ending. (Shenton & Pyner, 2014 with permission).

843



844
845 **FIGURE 1**
846

847 **FIGURE 2**
848


Heart Failure
Glutamate
ANG II
NMDAR
AT1
PSD95
nNOS
CAPON
CAPON
nNOS
PIN
CAPON
nNOS
PIN
Ub
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nNOS
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nNOS
nNOS
26S
Proteasome
nNOS
degradation
SNA

849
850

FIGURE 3

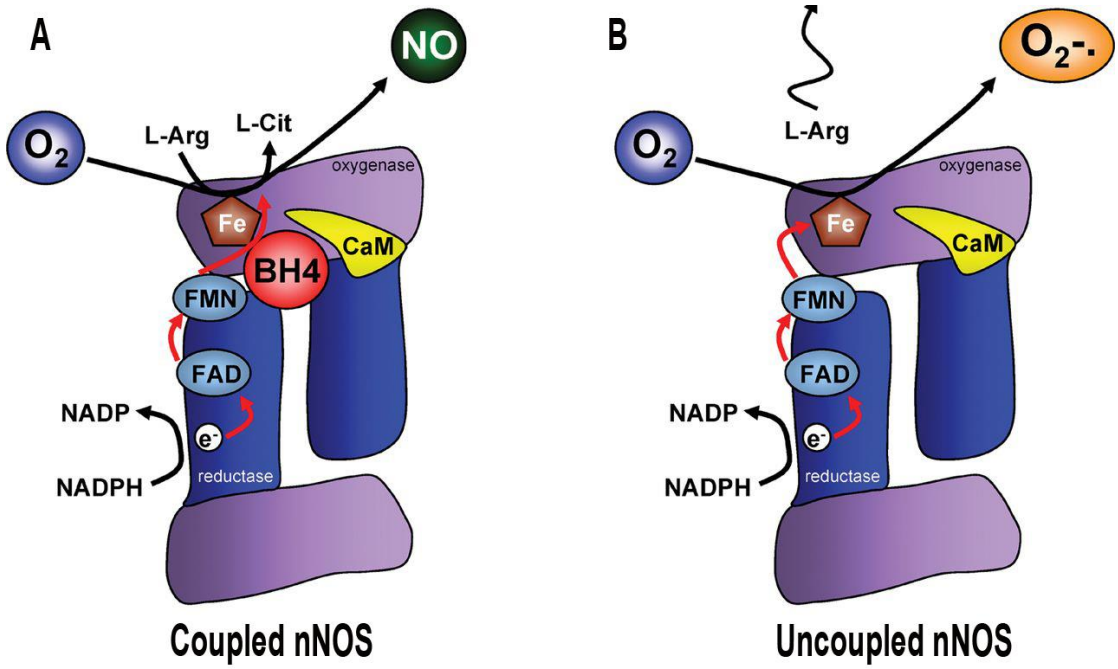
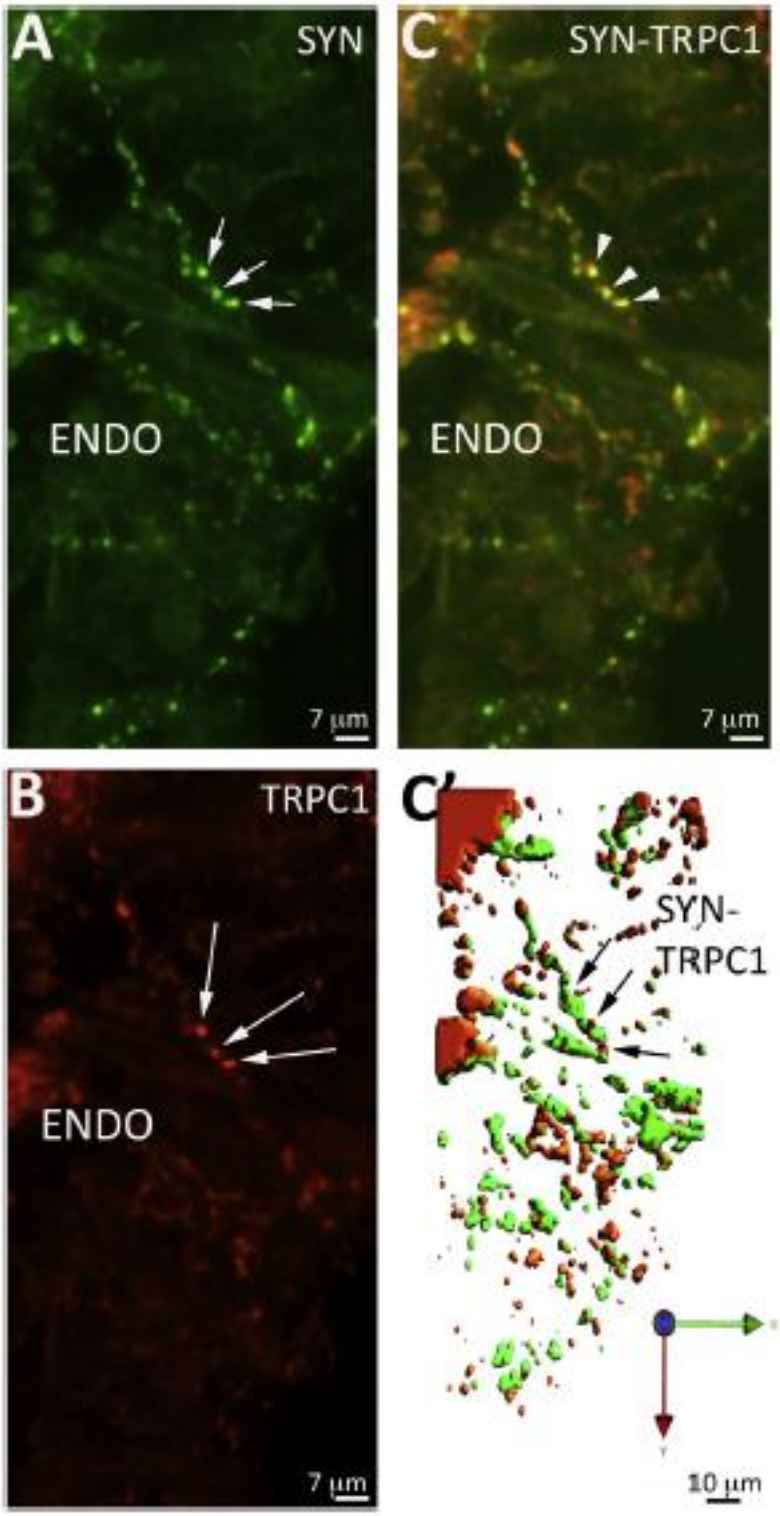
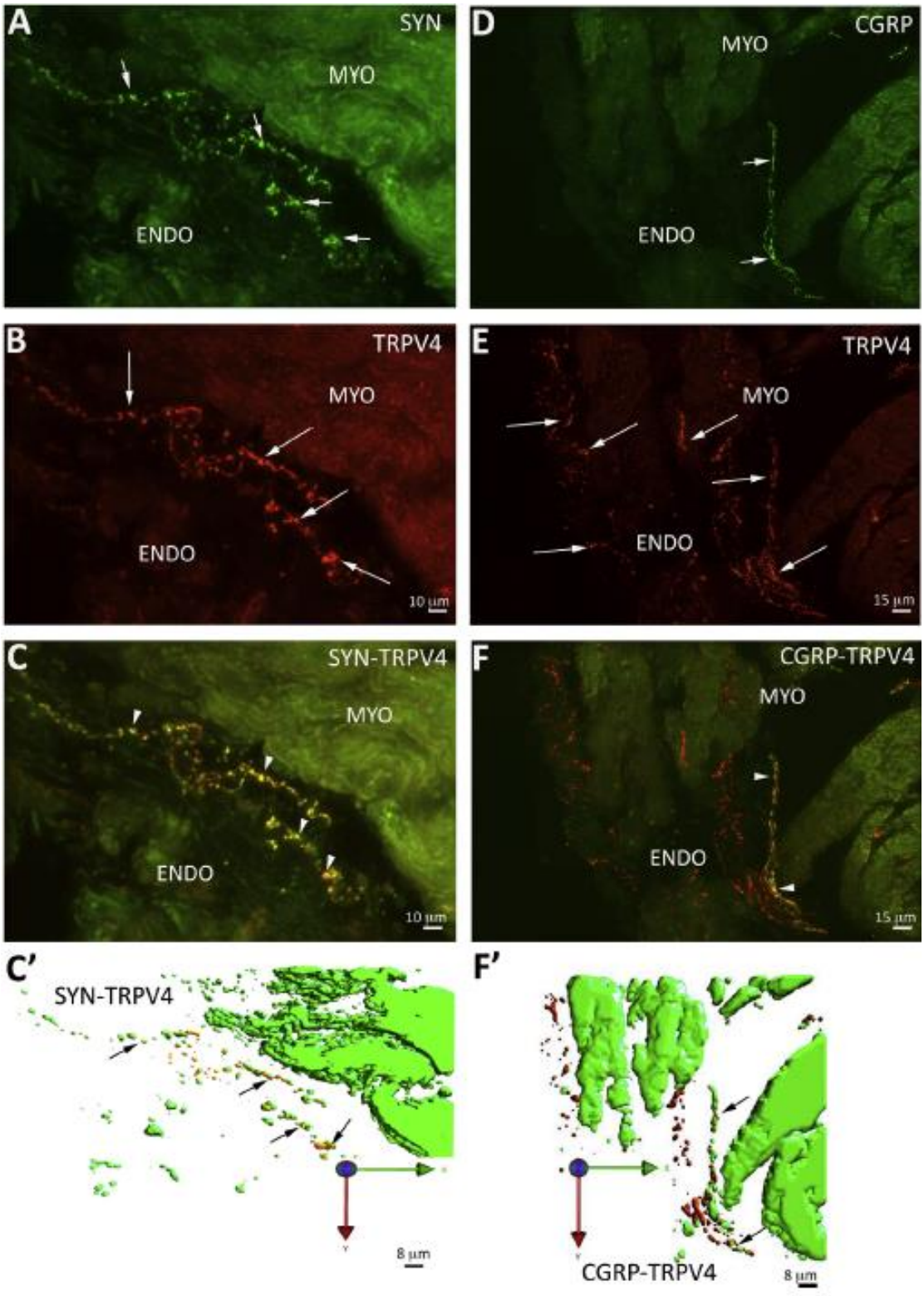

A
NO
O2
L-Arg
L-Cit
oxygenase
Fe
CaM
BH4
FMN
FAD
NADP
e-
NADPH
reductase
Coupled nNOS
B
O2-.
L-Arg
O2
oxygenase
Fe
CaM
FMN
FAD
NADP
e-
NADPH
reductase
Uncoupled nNOS

FIGURE 4


A
SYN
ENDO
7 µm
C
SYN-TRPC1
ENDO
7 µm
B
TRPC1
ENDO
7 µm
C'
SYN-
TRPC1
10 µm

857 **FIGURE 5**



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